Supporting Information

Molecular Detection of Bacterial Pathogens using Microparticle Enhanced Double-Stranded DNA Probes

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S1 Probe preparation

All probes and synthetic targets were synthesized by Integrated DNA Technologies Inc (Coralville, IA, USA) and other reagents are purchased from Sigma Inc. (St. Louis, MO, USA) unless otherwise specified. The buffer solution for the dsDNA probe contains 10 mM Tris-EDTA and 200 mM NaCl. In order to conduct the assay, the fluorophore and quencher probes were first mixed at a 1-to-3 ratio at 95°C for 5 minutes. The mixture is then allowed to cool down slowly to room temperature. The concentration of the probe (3.4 nM) is optimized for detecting low target concentrations.

S2. Assay protocol for detection of bacterial 16s rRNA

To perform the assay, the 100 µl sample was first centrifuged at 11,000 rpm for 5 min. Bacteria lysing was performed by the addition of 10 µl of 5 mg/ml of lysozyme from Invitrogen Inc. (Carlsbad, CA, USA) diluted into the 0.1% Triton X-100, 20 mM Tris-HCL and 2.0 mM EDTA for 10 min at room temperature. Then, 10 µl of 0.5 M NaOH was added to the solution and the mixture was incubated for 5 minutes at room temperature. 50 µl of the 4.7nM dsDNA probe including 0.5 mM phosphate buffer at pH 7.4 was added to the lysate to make the 3.4 nM final probe concentration. The mixture was incubated in a water bath incubator for 10 minutes at 65°C for hybridization. Then, the mixture was centrifuged at 13,000 rpm for 5 min and the pellet was discarded. In the case of microparticle enhanced dsDNA assay, streptavidin-coated 1.0 µm particles (4 µl of 0.001% w/v) with 2.7 nmol/mg binding sites capacity (which can bind up to 1.16×10^{15} molecules per particle) were added to the supernatant. The sample is incubated for 5 min at 37°C prior to fluorescence characterization.

S3. Fluorescence characterization

Fluorescence images of microparticles were obtained using an inverted epi-fluorescence microscope (TE2000-U, Nikon, Japan) with a digital charge-coupled device camera (Sensicam-QE, Cookecorp, Germany). Samples were loaded onto a cover slip and were illuminated using a mercury vapor lamp with a band-pass excitation filter at a wavelength of 515–560 nm. Fluorescence images were recorded using a dichromatic mirror at 580 nm and a long-pass filter at 590 nm. Representative fluorescence images of the microparticles at different target concentrations are shown in Figure 2. Quantitative analyses of the fluorescence images were performed using the NIH ImageJ software. The background of each sample was subtracted prior to data processing. In the microparticle experiment, at least 30 microparticles were selected for each sample to be analyzed.

S4 Statistics

All experiments were performed in triplicates and data are reported as mean ± standard deviation. Statistical analysis was conducted using Minitab Inc. (State College, PA, USA). To estimate the limit of detection (LOD) of the assays, non-linear regression is applied to analyze the data. The LOD values are determined by the two or three standard deviations above the background level representing approximately 95% and 99% probability levels respectively. The nonlinear calibration curve was used to convert the value into the corresponding target concentration. In this table, R^2 is above 0.9 in all experiments. P values are less than 0.05 and F-values are higher than F critical values in all cases. The results are summarized in Table 2.

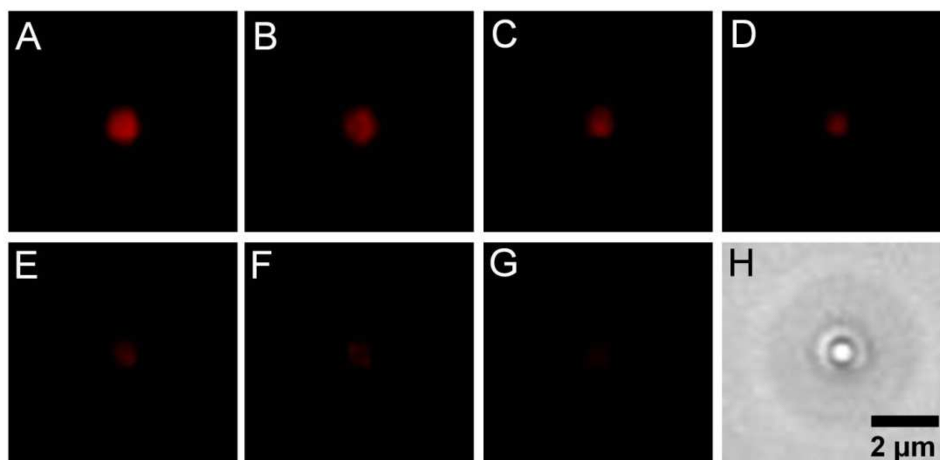

Figure S1. Fluorescence images of microparticles with different target concentrations: (A) 2×10^6, (B) 2×10^5, (C) 2×10^4, (D) 2×10^3, (E) 2×10^2, (F) 2×10^1, and (G) 0 cfu/ml. (H) Bright field image of a microparticle.

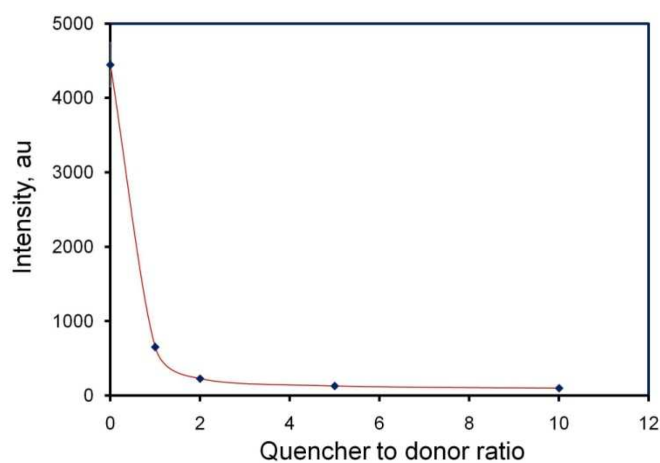

Figure S2. Background intensity at different quencher-to-fluorophore ratios. Different quencher concentrations are mixed with the fluorophore probe (3.4 nM) to determine the background intensity at different quencher-to-fluorophore ratios.

Table. S1 Sequence and modification of the DNA probes designed in this study

Probe Name	Lable	Sequence	Base Pair Number
***E. coli* Donor**	5' TAMRA^{TM}(NHS Ester)	5'-CTG CGG GTA ACG TCA ATG AGC AAA-3’	24
***E. coli* Donor-Bio**	5' TAMRA^{TM}(NHS Ester) 3' 3Bio	5'-CTG CGG GTA ACG TCA ATG AGC AAA-3’	24
***E. coli* Quencher**	3' Iowa Black RQ^{TM}	5'-CGT TAC CCG CAG- 3'	12
***E. coli* Target**	Unlabeled	5'-TTT GCT CAT TGA CGT TAC CCG CAG- 3'	24

Table.2 Comparison of the limit of detection of the assay under different conditions

Assay	Target	R-Sq	F value	F critical	P	LOD (99%)	LOD (95%)
dsDNA with beads	***E. coli* (cfu)**	0.935	28.97	10.13	0.033	13 CFU	**8 CFU**
dsDNA only	***E. coli* (cfu)**	0.999	1362.2	10.13	0.001	14450 CFU	**10520 CFU**
dsDNA with beads	**Synthetic target (fM)**	0.959	47.03	10.13	0.021	5.8 fM	**4.5 fM**
dsDNA only	**Synthetic target (pM)**	0.987	1123.3	10.13	0.002	6.2 pM	**4.8 pM**